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CYTOKINE REGULATION OF HOST DEFENSE AGAINST PARASITIC GASTROINTESTINAL NEMATODES: Lessons from Studies with Rodent Models*

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ABSTRACT

Studies with rodents infected with *Trichinella spiralis*, *Heligmosomoides polygyrus*, *Nippostrongylus brasiliensis*, and *Trichuris muris* have provided considerable information about immune mechanisms that protect against parasitic gastrointestinal nematodes. Four generalizations can be made: 1. $CD4^+$ T cells are critical for host protection; 2. IL-12 and IFN-γ inhibit protective immunity; 3. IL-4 can: (*a*) be required for host protection, (*b*) limit severity of infection, or (*c*) induce redundant protective mechanisms; and 4. Some cytokines that are stereotypically produced in response to gastrointestinal nematode infections fail to enhance host protection against some of the parasites that elicit their production. Host protection is redundant at two levels: 1. IL-4 has multiple effects on

the immune system and on gut physiology (discussed in this review), more than one of which may protect against a particular parasite; and 2. IL-4 is often only one of multiple stimuli that can induce protection. Hosts may have evolved the ability to recognize features that characterize parasitic gastrointestinal nematodes as a class as triggers for a stereotypic cytokine response, but not the ability to distinguish features of individual parasites as stimuli for more specific protective cytokine responses. As a result, hosts deploy a set of defense mechanisms against these parasites that together control infection by most members of that class, even though a specific defense mechanism may not be required to defend against a particular parasite and may even damage a host infected with that parasite.

INTRODUCTION

Medical and Economic Importance of Gastrointestinal Nematode Infections

Gastrointestinal roundworm parasites, including those within the genera Necator, Ancylostoma, Ascaris, Trichuris, and Strongyloides, infect approximately one billion people worldwide and are believed to cause approximately one million deaths annually (1, 2). Children in developing countries are particularly likely to be infected by gastrointestinal nematodes; in some endemic areas the prevalence by age 10 approaches 100% (3). Infections tend to be chronic and reinfection rates high. Chronic nematode infections can be particularly damaging to children, causing growth retardation and impaired cognitive function in severely infected individuals (4, 5). In addition to their direct pathogenic effects, gastrointestinal worm infections can also predispose to secondary bacterial and protozoan infections (6, 7). The economic damage produced by the detrimental effects of gastrointestinal nematode parasites on livestock production worldwide adds to the misery they cause through human infection.

Although primary health care and effective public sanitation can successfully eliminate human gastrointestinal parasitism, immunological intervention may promote control in situations where gastrointestinal parasitism remains endemic and intractable. Successful immunization procedures may be more cost-effective and practical than other forms of therapy and could reduce consumer concerns over antihelmintic drug residues in livestock. Inasmuch as infections with small numbers of worms are generally well tolerated, immunity need not be complete to protect against symptomatic disease. Furthermore, an immune response that decreases worm fecundity will have important epidemiological significance even if it fails to decrease the number of adult worms harbored by an individual host because it will decrease the spread of infection in a community.

The ability of immune responses to control gastrointestinal nematode infections has been demonstrated in experimental animals by correlations between host resistance and the expression of MHC and non-MHC genes that regulate

immune responses (8) and by protective vaccination (see below). In humans infected with some gastrointestinal nematodes such as *Trichuris trichiura*, a close relative of the mouse parasite *Trichuris muris*, immune regulation of infection is compatible with observations that the intensity of a reinfection following drug treatment usually resembles that of the initial infection (9, 10). Because the type of immune response made to a parasite is as important as the magnitude of the response in controlling infection, discovery of procedures that induce the proper type of response will be a critical requirement for successful immunological control of gastrointestinal nematode infections.

Cytokine Regulation of Host Protection Against Parasite Infections

Understanding of the mechanisms that are important for regulation of antiparasite immune responses was greatly advanced by two discoveries: First, $CD4^+$ T cells differ in the patterns of cytokines that they express. Two polar $CD4^+$ T cell groups were described: Th1 cells that secrete interferon (IFN)-γ, interleukin (IL)-2, and lymphotoxin, but not IL-4, IL-5, IL-9, or IL-10; and Th2 cells that secrete IL-4, IL-5, IL-9, and IL-10, but not IL-2, IFN-γ, or lymphotoxin (11). And second, host survival can depend upon the set of cytokines that are produced in response to an infectious agent. Initial studies, with mice inoculated in their footpads with *Leishmania major*, showed that C57BL/6 and C3H/HeN mice, which make a predominantly Th1 response, resolve infections, whereas BALB/c mice, which make a predominantly Th2 response, develop a chronic, and eventually lethal, systemic infection. IFN-γ and IL-4 were demonstrated to be critical cytokines in regulating host responses to *L. major* infection: Antibody neutralization of IFN-γ leads to lethal *L. major* infection in the normally resistant strains, while treatment with anti-IL-4 mAb allows normally susceptible BALB/c mice to resolve an *L. major* infection. An additional cytokine, IL-12, promotes resistance to *L. major* by stimulating IFN-γ production and inhibiting IL-4 production (12).

The effects of IFN-γ and IL-4 on macrophage production of inducible NO synthase (iNOS), the enzyme that catalyzes macrophage production of NO, are largely responsible for their regulation of host recovery from *L. major* infection: Macrophage killing of ingested *L. major* depends upon NO production, and iNOS is induced by IFN-γ and suppressed by IL-4 (13). Subsequent studies demonstrated connections between a Th1 response and host defense against several intracellular parasites, bacteria, and viruses, including *Toxoplasma gondii* (14), *Plasmodium* sps. (15, 16), *Cryptosporidium parvum* (17, 18), *Listeria monocytogenes* (19), and murine cytomegalovirus (20), although control of these infections may be mediated by Th1-associated cytokine stimulation of NK activity, CTL function, and the production of cytotoxic antibodies, in addition to iNOS induction (21–23).

Observations that the cytokines that are produced by Th1 cells (type 1 cytokines) are involved in host control of so many infectious agents made researchers question whether the cytokines that are produced by Th2 cells (type 2 cytokines) have any host-protective role. One suggestion was that type 2 cytokines function predominantly to limit inflammation that is induced by type 1 cytokines. Experimental data support the view that this is one function of some type 2 cytokines. IL-10–deficient mice develop inflammatory bowel disease and have increased susceptibility to septic shock (24, 25). However, it is arguable that IL-10 should not be considered a type 2 cytokine because IL-10 production does not correlate particularly well with production of other type 2 cytokines in pathogen-infected animals (26, 27). IL-4 also has some anti-inflammatory effects: Although mice that lack a functional IL-4 gene do not develop spontaneous inflammatory disease (28, 29), they are more susceptible than normal mice to a TNF-associated wasting syndrome that is caused by infection with *Schistosoma mansoni* (E Pearce, personal communication). Other type 2 cytokines such as IL-5 have no known anti-inflammatory effects (30), and IL-4 and IL-5 themselves induce an inflammatory response that is characterized by mastocytosis, IgE production, and eosinophilia (31, 32).

An additional possibility is that control of some infectious agents is regulated differently from that of the intracellular parasites mentioned above, so that type 2 cytokine responses may limit rather than exacerbate disease. This possibility was investigated in studies of mice infected with gastrointestinal nematodes, which characteristically induce type 2 cytokine responses with eosinophilia, increased IgE levels, and mucosal mastocytosis (33). This paper reviews the evidence for type 2 cytokine control of mouse and rat infections with four such parasites: (i) *Nippostrongylus brasiliensis* and (ii) *Heligmosomoides polygyrus* (i.e. Nematospiroidies dubius), from the superfamily Heligmosomatoidea, which generally produce acute and chronic infections, respectively, in rodents, and have life cycles similar to trichostrongyle parasites that infect the small intestines of humans and livestock; and (iii) *Trichuris muris* and (iv) *Trichinella spiralis* from the superfamily Trichineloidea, which interact, respectively, with gut epithelial cells in the large intestine of mice and in the small intestine of more than 100 mammalian species, including humans, livestock, and rodents.

PARASITIC GASTROINTESTINAL NEMATODES USED FOR ANIMAL EXPERIMENTS

Two to four hours after ingestion of muscle tissue that contains the encysted first stage larvae of *T. spiralis*, larvae ecdyse in the host stomach and enter duodenal or jejunal epithelium. Larvae mature into adults and mate in the next 36 hours.

Adult worms induce syncytium formation and reside within intestinal epithelial cells. Female worms release larvae, beginning 4–7 days after ingestion. Larvae enter intestinal lymphatics or mesenteric venules and migrate throughout the host body, settling most heavily in host striated muscle, where they start to encapsulate by 17–21 days after ingestion. Adult worms can remain in the gut of large mammals such as pigs and humans for several weeks, but generally reside in rodent intestines for less than 2 weeks (34, 35).

H. polygyrus is a nematode parasite, native to mice, that generally establishes chronic infections and lives only in the gut of its mammalian host. The infective stage is free-living as a third-stage larva in a second molt cuticle. Parasitic third-stage larvae enter the wall of the anterior small intestine within the first 24–72 h after ingestion. They reside in the circular muscle layer of the muscularis externa, where they feed on host tissues and develop until they exit into the gut lumen 8 days after oral inoculation (36). Once in the gut lumen, they rapidly mature into adults, feed on host intestinal mucosa, and continue to live, in most mouse strains, for several months (37). Although primary infections are chronic, challenge inocula may be eliminated by immune hosts in a much shorter period of time (38–40).

N. brasiliensis, which is naturally a rat nematode parasite, has been adapted to the mouse for experimental purposes (41). Infective larvae are free-living in the third stage. Mouse-adapted strains of *N. brasiliensis* penetrate (or are injected through) the host skin and, 24–48 h later, migrate to the lungs, where an inflammatory response is induced that is characterized by pulmonary eosinophilic granulomas. Larvae are coughed up and swallowed 48–72 h after inoculation, and mature in the jejunum into egg-laying adults by 5–6 days after inoculation. Adult worms are expelled, damaged but still alive, from the gut of immunocompetent rodents less than 2 weeks after inoculation (42). Challenge inocula of *N. brasiliensis* larvae reach the gut in reduced numbers and produce eggs transiently if they produce eggs at all (43, 44). Thus, this parasite can be used as a model for short-lived infections that have a systemic as well as a gastrointestinal phase in which host defense mechanisms cause expulsion without killing the parasite.

The whipworm *Trichuris muris* is a nematode with an infective first-stage larva contained in an environmentally resistant egg that enters mice by ingestion and parasitizes the mouse cecum and colon, where its head and part of its filamentous anterior region embed in, and digest, host mucosal epithelium. It causes chronic infections in some mouse strains but is expelled from other strains before egg-laying adults can develop (45). Differences in the course of *T. muris* infection among inbred mouse strains reflect the spectrum of infection that is seen in outbred mice infected with the same parasite and in humans infected with the closely related parasite *T. trichiura* (46, 47).

T CELL AND CYTOKINE REGULATION OF HOST-PROTECTIVE IMMUNITY

General Considerations

Given the diverse life cycles of the four parasites discussed here, it is not surprising that no single immunological mechanism of host protection can be identified that is as pervasive as the IL-12/IFN-γ/iNOS pathway that protects hosts against many intracellular parasites. At least four generalizations are, however, apparent:

PROTECTIVE IMMUNITY AGAINST GASTROINTESTINAL NEMATODE PARASITES IS CD4$^+$ T CELL-DEPENDENT This fact has been demonstrated in studies in nude (congenitally athymic) mice and rats, as well as in studies of mice treated with a cytotoxic anti-CD4 mAb (48–55). Anti-CD4 mAb, but not anti-CD8 mAb, treatment prevents expulsion of *N. brasiliensis* and promotes parasite egg production for as long as the mAb treatment is maintained. Mice are still protected against reinfection, however, if anti-CD4 mAb is injected only at the time of the challenge infection, even though this treatment suppresses polyclonal antibody responses to the challenge infection (50). Thus, CD4$^+$ T cells are more likely to induce host-protective mechanisms than to directly participate in worm elimination.

As is true for *N. brasiliensis* infections, anti-CD4 mAb treatment prolongs a primary infection of normally resistant mouse strains with *T. muris* and allows larvae to develop into fecund adults (53). CD4$^+$ T cell dependence of protective immunity against *T. spiralis*, in contrast, is best demonstrated during challenge infections. Previously infected normal rats expel most ingested or orally inoculated *T. spiralis* larvae in less than 1 h; this rapid expulsion phenomenon is not observed in nude rats (54). Furthermore, CD4$^+$ T cells have been shown in transfer experiments with mice to mediate protection against a challenge *T. spiralis* infection (55). CD4$^+$ T cell–dependent immunity to *H. polygyrus* during a primary infection is demonstrated by the increased egg production that results from treating hosts with anti-CD4 mAb. Immunity is more obvious during challenge infections with *H. polygyrus* in previously infected, drug-cured BALB/c mice. Challenge infections are usually much milder than primary infections, with reduced adult worm survival and little egg production by surviving worms. Treatment with anti-CD4 mAb at the time of the challenge infection completely blocks host immunity (51).

IL-12 AND IFN-γ PROMOTE THE SURVIVAL OF GASTROINTESTINAL NEMATODE PARASITES This has been particularly well demonstrated in mice infected with either *T. muris* or *N. brasiliensis*. Mouse strains that produce a strong

IFN-γ response to a primary *T. muris* infection, unlike strains that produce a predominantly IL-4 response, develop chronic infections with this parasite. Treatment with a neutralizing anti-IFN-γ mAb at the time of *T. muris* inoculation results in expulsion of larvae before they can develop into fecund adults (56). In contrast, treatment of normally resistant mouse strains with IL-12 during the second week of *T. muris* infection causes an IFN-γ-dependent increase in host susceptibility that allows larvae to mature into egg-laying adults (AJ Bancroft, J Sypek, KJ Else, RK Grencis, personal communication). Similarly, treatment of *N. brasiliensis*–infected mice with IFN-γ or IL-12, starting at the time of parasite inoculation, enhances egg production severalfold and prolongs the course of infection (57). The effects of IL-12 on the course of an *N. brasiliensis* infection depend predominantly on the production of IFN-γ, because they are minimal in anti-IFN-γ mAb-treated mice and in mice that lack a functional gene for IFN-γ (57; JF Urban Jr, FD Finkelman, unpublished data). IL-12 treatment during a primary *N. brasiliensis* infection retards, but does not permanently inhibit, Th2 cytokine responses and worm expulsion during a challenge infection, although worm expulsion will continue to be suppressed during a challenge infection if IL-12 treatment is continued. Effects of IL-12 treatment on responses by BALB/c mice to an *N. brasiliensis* infection differ from effects on responses to an *L. major* infection in that a type 2 cytokine response develops and adult worms are expelled once IL-12 treatment is discontinued during the *N. brasiliensis* infection (57; JF Urban Jr, FD Finkelman, unpublished data), but a type 1 response and host protection continue when IL-12 treatment is terminated during the *L. major* infection (12).

Initial studies with mice infected with *T. spiralis* suggested that IFN-γ might be important for host protection. During a primary infection, adult worms were expelled from the gut more rapidly in AKR mice than in MHC-identical B10.BR mice. Infected AKR mice developed higher levels of IFN-γ-dependent IgG2a anti-*T. spiralis* antibodies than did infected B10.BR mice, and in vitro anti-CD3 mAb stimulation of mesenteric lymph node cells from AKR mice induced more IFN-γ secretion than did similar stimulation of cells from B10.BR mice (58). However, subsequent studies demonstrated no inhibitory effect of anti-IFN-γ mAb treatment on worm expulsion (JF Urban Jr, HR Gamble, FD Finkelman, unpublished data; DL Wassom, personal communication). Furthermore, rapid expulsion of *T. spiralis* larvae by rats can be transferred with immune T cells that secrete high levels of IL-4 and low levels of IFN-γ, but not by T cells that secrete high levels of both IL-4 and IFN-γ (59). Finally, the ability of IgE from previously infected rats to transfer the rapid expulsion response (60, 61) is also consistent with a suppressive effect of IFN-γ, inasmuch as this cytokine suppresses IgE responses (62).

IL-4 PROMOTES PROTECTIVE IMMUNITY AGAINST GASTROINTESTINAL NEMATODE PARASITES The host-protective effects of IL-4 have been most prominently demonstrated in mice infected with *T. muris* and *H. polygyrus*. Anti-IL-4 or anti-IL-4R mAb block host immunity to a challenge *H. polygyrus* infection, as demonstrated by increased adult worm survival and egg production (63). Similarly, treatment with anti-IL-4R mAb [which blocks the effects of IL-13 as well as those of IL-4 (RA Morawetz, L Gabriele, LV Rizzo, N Noben-Trauth, R Kühn, K Rajewsky, W Müller, TM Doherty, F Finkelman, RL Coffman, HC Morse III, "IL-4-independent immunoglobulin class switch to IgE in the mouse," submitted for publication)] causes normally resistant BALB/k mice to develop chronic infections with *T. muris* (56). IL-4 dependence of host resistance to these two parasites may differ, however, in that mice that lack a functional gene for IL-4 fail to develop immunity to *H. polygyrus* (JF Urban Jr, FD Finkelman, unpublished data), but still can expel *T. muris* (A Bancroft, KJ Else, RM Grencis, personal communication). This suggests that either the chronic absence of IL-4 can lead to the development of an alternative mechanism that limits worm survival in *T. muris*-infected, but not in *H. polygyrus*-infected, mice, or that IL-13 can substitute for IL-4 in promoting *T. muris* expulsion, but not *H. polygyrus* expulsion.

The important role for IL-4 in controlling *H. polygyrus* and *T. muris* infections has also been demonstrated by experiments in which mice with chronic infections were treated with a formulation of IL-4 that has a long in vivo half-life (IL-4 complexes, prepared by mixing IL-4 and a neutralizing anti-IL-4 mAb at a 2:1 molar ratio, so that the antibody acts as a carrier protein that protects the cytokine from degradation and excretion) (64). Treatment with IL-4 that has been complexed in this way (IL-4C) cures even established primary *T. muris* and *H. polygyrus* infections (56, 65).

The role of IL-4 in host protection against *N. brasiliensis* and *T. spiralis* is less straightforward. *N. brasiliensis* is expelled normally, or with only a slight delay, in anti-IL-4 mAb-treated mice and in mice that lack a functional gene for IL-4 (17, 66). However, treatment with IL-4C terminates the chronic *N. brasiliensis* infections that develop in anti-CD4 mAb-treated mice or SCID mice (65). Inasmuch as IL-4 production is strongly induced in immunocompetent mice by an *N. brasiliensis* infection (67), it seems likely that this cytokine stimulates a host-protective mechanism that is normally redundant but that becomes critical when other $CD4^+$ T cell–dependent mechanisms are blocked.

Anti-IL-4R mAb treatment of *T. spiralis*-infected mice has a modest, but reproducible disease-exacerbating effect [increased survival of adult worms and increased numbers of muscle larvae (JF Urban Jr, HR Gamble, FD Finkelman, unpublished data)]. Reports of increased numbers of muscle larvae in IgE-depleted, *T. spiralis*-infected rats and of transfer of rapid expulsion with purified

IgE antibody (60, 61, 68) also support a protective role for IL-4, because nematode infection-induced IgE production is IL-4-dependent (69).

NOT ALL NEMATODE INFECTION-ASSOCIATED CYTOKINES ARE CONSISTENTLY HOST-PROTECTIVE IL-5 is a case in point. All of the gastrointestinal nematode infections discussed here stimulate eosinophilia and increased production of IL-5, a cytokine that stimulates eosinophil production and activation (70–72). Because eosinophils kill some parasite larvae in vitro (73), experiments have examined the in vivo role of eosinophils or IL-5 in host control of nematode infections. Most of the results of these studies have been negative. Although treatment of *T. spiralis*-infected rats with a polyclonal anti-eosinophil antiserum was reported to increase numbers of muscle larvae (74), *T. spiralis*-infected mice that were treated with anti-IL-5 mAb to prevent eosinophil responses did not have increased susceptibility to infection (75). Similarly, anti-IL-5 mAb has no effect on control of *T. muris*, *H. polygyrus*, or *N. brasiliensis* infections in mice, even though it prevents blood and tissue eosinophilia in mice infected with any of these parasites (63, 72; KJ Else, RL Grencis, personal communication). In contrast, increased killing of *N. brasiliensis* larvae while they reside in the lungs has been reported in transgenic mice that express increased amounts of IL-5 (76). Additional evidence exists for IL-5-dependent killing of other helminths outside of the gut: *Angiostrongylus cantonensis* larvae in mouse brain (77); *Strongyloides venezuelensis* larvae in mouse lung (78). These observations suggest that eosinophils may contribute less to host protection against parasites that reside within the gut than to protection against parasites residing in other host organs.

Physiology of Worm Expulsion

Evidence that IL-4 has a central role in the control of *H. polygyrus* and *T. muris* infections, and can contribute to the control of *N. brasiliensis* and *T. spiralis* infections, suggests that the molecular and physiological effects of this cytokine may contribute to control of infection. Well-established effects of IL-4 that might influence worm expulsion include stimulation of IgE responses (79) [and in the mouse, IgG1 responses (80)], stimulation of mucosal mastocytosis (64, 66), promotion of T cell type 2 cytokine responses (81), stimulation of T cell growth (82–85), and enhancement of expression of VCAM-1 [the endothelial cell receptor for the integrin VLA-4, which is involved in the migration of macrophages, lymphocytes, and eosinophils across venous high endothelium (86, 87)]. The best evidence for the involvement of at least some of these mechanisms comes from studies of rapid expulsion of *T. spiralis* from the gut of immune rats.

RAPID EXPULSION OF *T. SPIRALIS* Most *T. spiralis* larvae ingested by a previously infected rat are expelled in less than 1 h, before they can embed in the

intestinal mucosa. The small percentage of larvae that embed in the mucosa resist rapid expulsion and remain within the host for several days (88). The ability to rapidly expel larvae is most easily transferred to naive rats with a combination of lymphoid cells and serum from immune rats, although transfer of immune antiserum alone, or IgG1 or IgE fractions of immune serum, has enabled rapid expulsion in some studies (60, 61, 89, 90). Mast cell involvement in rapid expulsion is suggested by the presence of increased serum levels of rat mast cell protease II at the time of expulsion (91). Increases in the mast cell products leukotriene (LT)C4 (which causes smooth muscle contraction, increased vascular permeability, and mucus hypersecretion) and LTB4 (which recruits and activates inflammatory cells) are seen 30–60 min after larval challenge in the mucosal tissue and in the secretions of the proximal small intestine (92). Release of histamine, 5-hydroxytryptamine (serotonin), and prostaglandin (PG)E2 also occurs (93).

Within the same time period, changes are seen in gut myoelectric patterns in vivo (94) that suggest stimulation of gut smooth muscle contraction. In vitro studies have, in fact, demonstrated that exposure of the small intestine of *T. spiralis*-immune rats to *T. spiralis* antigen increases smooth muscle contractility, and that this response is dependent upon mast cell activation and 5-hydroxytryptamine, but not on histamine or prostaglandins (95). Consistent with this observation, the rapid rejection response is blunted in vivo when rats are treated with 5-hydroxytryptamine S2 and S3 receptor antagonists (ketanserin and MDL-72222, respectively), but not when they are treated with type 1 histamine (H1) receptor antagonists or with inhibitors of cyclooxygenase or 5-lipoxygenase (96).

Changes in small intestine fluid dynamics are also associated with rapid expulsion of *T. spiralis*. Decreased fluid absorption is observed 30 min after a second infection (97), and a mast cell–dependent, chloride ion–dependent increase in short circuit current (a measure of net ion flux) is rapidly induced by in vitro exposure of immune gut to *T. spiralis* antigen (60, 98). Intra-arterial perfusion of serotonin into non-immune rats causes fluid secretion and reduced worm establishment, and a combination of serotonin S2 and S3 receptor antagonists reduces fluid secretion and promotes worm establishment (96). Taken together, these observations suggest that rapid expulsion of *T. spiralis* larvae by immune rats results from an anaphylactic reaction that is localized to the gut and is caused by antibody (IgE or IgG)-mediated mast cell degranulation, with 5-hydroxytryptamine playing a dominant role and smooth muscle contraction and increased fluid secretion serving as possible mechanisms that inhibit larval penetration into the gut mucosa. No firm evidence is available, however, to demonstrate that smooth muscle contraction and/or increased fluid secretion are essential for rapid expulsion. In fact, inhibition of increased fluid secretion by

treatment of mice with H1 receptor antagonists and cyclooxygenase inhibitors has no suppressive effect on worm expulsion. Furthermore, induction of enhanced fluid secretion by PGE2, cholera toxin, and hypertonic Krebs-mannitol solution does not reduce larval numbers (99).

PRIMARY INFECTIONS WITH *T. SPIRALIS* During a primary infection of rats with *T. spiralis*, larvae penetrate into the small bowel mucosa and develop into adults, which are expelled over a period of weeks, rather than minutes (35). Nevertheless, some of the same changes in gut physiology that are observed during the rapid expulsion phenomenon develop during a primary infection: gut motility, jejunal radial and longitudinal smooth muscle layer thickness, and longitudinal smooth muscle contractility all increase during infection (95, 100, 101). There is also a marked change in the pattern of peristalsis: Normal coordinated contractions decrease, with a reduction in electrical slow wave activity and spike potential frequencies. However, a migrating action potential complex that rapidly sweeps through the bowel is observed and may have the effect of expelling loosely attached parasites (102). Furthermore, by five days after the initiation of a primary infection, small intestinal fluid dynamics have changed from net absorption to net secretion (97). Thus, it is likely that the same physiological processes that are associated with rapid expulsion develop more chronically during a primary infection and may have a role in limiting adult worm survival. Although the stimulatory effects of IL-4 on mast cells and IgE production, which are strongly associated with rapid expulsion, suggest that IL-4 has an important role in the induction of host immunity against *T. spiralis* in the rat, the lack of neutralizing anti-rat IL-4 antibodies or IL-4-deficient rats has prevented direct testing of this hypothesis.

Studies in mice complement the rat studies. Although the classic rapid expulsion phenomenon probably does not exist in mice (52), mouse studies of host-protective mechanisms against *T. spiralis* have been facilitated by the greater availability of immunological reagents that work in mice than those that work in other experimental species. Studies with anti-IL-4 receptor mAb have directly demonstrated that IL-4 (or IL-13) has a role in limiting *T. spiralis* infections (JF Urban Jr, HR Gamble, FD Finkelman, manuscript in preparation). Studies in which infected mice have been treated with IL-9, a cytokine that mimics or enhances some of the effects of IL-4 (103, 104), have demonstrated that this cytokine also can accelerate *T. spiralis* expulsion (HC Faulkner, RK Grencis, personal communication). Involvement of mast cells in mouse expulsion of *T. spiralis*, including IL-9-accelerated expulsion, has been demonstrated by experiments in which mucosal mastocytosis was blocked and expulsion delayed or prevented by treatment with anti-*c-kit* mAb (105; HC Faulkner, RK Grencis, personal communication).

STUDIES WITH *H. POLYGYRUS*-INFECTED MICE Studies using mice infected with this parasite and uninfected mice that have been treated with IL-4C have demonstrated IL-4 involvement in the stimulation of physiological changes that are associated with worm expulsion, but they have not yet identified a unique mechanism by which IL-4 induces worm expulsion. Increased small intestine smooth muscle contractility is one such physiological change that is observed during a second *H. polygyrus* infection, in which immune system control of infection is marked, but not during a primary infection in which immune system control of infection is less evident (106, 107). Increased small intestine smooth muscle contractility can by blocked by treating mice during a second *H. polygyrus* infection with anti-IL-4 receptor mAb, and it can be induced in uninfected mice by treating them over a period of 6 days with IL-4C. The IL-4C-induced increases are blocked by an inhibitor of LTD4 and are not observed in SCID mice, in 5-LO-deficient mice, or in W/W^v mice (107), which have defective *c-kit* expression that blocks mast cell development (108–110). Although IL-4 induces an increase in intestinal smooth muscle responsiveness, it neither speeds nor retards transit through the gut (107). In fact, as has been described in *T. spiralis*-infected rats (102), BALB/c mice infected for a second time with *H. polygyrus* lose normal peristaltic activity, so that ingested material is evenly, rather than segmentally, distributed in the gut. This effect is $CD4^+$ T cell dependent but is neither IL-4-dependent nor reversible by treatment with IL-4C (107). Thus, if the changes in smooth muscle reactivity that are induced by IL-4C have a role in *H. polygyrus* expulsion, they probably mediate increased gut spasticity that might limit the access of worms to their food source, the gut mucosa (111), rather than create a caudally directed, irresistible flow of gut contents.

Both IL-4C and a second *H. polygyrus* infection also induce changes in small bowel fluid dynamics. In BALB/c mice, IL-4C treatment and a second *H. polygyrus* infection both increase small intestinal permeability and decrease the ability to absorb fluid from the small intestine in response to glucose. Both responses are reversed in *H. polygyrus*-infected mice by treatment with anti-IL-4 receptor mAb. Neither response is seen in IL-4C-treated or *H. polygyrus*-infected W/W^v mice. IL-4C-treated SCID mice, which lack B and T lymphocytes, develop increased intestinal permeability but do not demonstrate a decreased fluid absorption response to glucose. In addition to increased small intestinal permeability and decreased fluid absorption in response to glucose, both IL-4C treatment and a second *H. polygyrus* infection increase the secretory response to PGE2. This elevated secretory response is blocked in *H. polygyrus*-infected mice by anti-IL-4 receptor mAb, is totally dependent on neural regulation in *H. polygyrus*-infected mice (but not in uninfected, IL-4C-treated mice), and occurs in SCID mice but not in W/W^v mice (C Sullivan,

JF Urban Jr, SC Morris, FD Finkelman, T Shea-Donohue, manuscript in preparation). Thus, the net effects of exogenous or endogenously produced IL-4 in *H. polygyrus*-infected mice most likely increase the fluid content of the gut lumen by increasing secretion and decreasing absorption. This view is reinforced by the direct demonstration of increased fluid in the jejunum of IL-4C-treated, uninfected mice (C Sullivan, JF Urban Jr, SC Morris, FD Finkelman, T Shea-Donohue, manuscript in preparation).

The absence of IL-4 effects on intestinal smooth muscle contractility and on intestinal fluid dynamics in W/W^v mice suggests that intestinal mucosal mast cells, which increase in number in response to IL-4C treatment, have a role in mediating these effects. This interpretation is made less certain, however, by the presence of abnormalities in W/W^v mice, besides the near total absence of mast cells. These additional defects include an absence of the interstitial cells of Cajal, which regulate peristalsis by acting as intestinal pacemakers to smooth muscle (112), and a lack of intraepithelial $\gamma\delta$ T cells (113).

An additional uncertainty is whether the physiological changes that are induced by IL-4C treatment are related to the mechanisms by which endogenously produced IL-4 limits worm survival and egg production during a second *H. polygyrus* infection. Evidence that IL-4 has a critical role in host protection against *H. polygyrus* comes from two observations: 1. The decreased fecundity and adult worm survival that typify a second infection are blocked by treatment with anti-IL-4 or anti-IL-4 receptor mAb (63); and 2. IL-4C treatment decreases egg production and causes worm expulsion during a primary infection (65). The mechanisms by which endogenously produced IL-4 and treatment with exogenous IL-4C promote host protection may differ, and this possibility is aggravated by uncertainty about the stage of infection against which the host-protective effects of IL-4 operate. Different investigators, working with different mouse strains and strains of *H. polygyrus*, have come to different conclusions about the life-cycle stage of *H. polygyrus* that is the target of the immune response during a challenge infection. One group has reported that immunity is directed entirely against larvae that are developing within the gut wall and is manifested by destruction of developing larvae (106). A second group has reported that immune mechanisms arrest larval development within the gut wall, without initially killing the larvae (114), and that larvae are killed or expelled shortly after emerging into the gut lumen (115). A third group has reported that immunity limits reinfection by preventing the initial penetration of larvae into the gut wall, by killing larvae that reside in the gut wall, and by killing worms shortly after they have emerged into the gut lumen (116). These disparate findings all suggest that IL-4-dependent, host-protective mechanisms operate relatively early in an infection, and the findings are consistent with the observation that adult worms that remain present weeks after an initial

inoculation with *H. polygyrus* are not expelled by the immune response to a challenge infection, even though most larvae from the challenge inoculum are destroyed or expelled (106, 114).

In contrast to these observations, treatment with exogenous IL-4 during a primary infection is directed against adult worms: IL-4C treatment during the encysted larval stage has little effect, while IL-4C treatment that commences after worms have entered the gut lumen first decreases egg production and then, after 6–9 days, causes worm expulsion (65). Thus, even if a major host-protective effect of IL-4 is the induction of changes in gastrointestinal physiology that expel worms shortly after they transit from the gut wall to the gut lumen, it is likely that some host-protective events that depend on endogenous IL-4 production during a challenge infection differ from host-protective events that are induced by IL-4C treatment during a primary infection.

Another unresolved issue is whether the changes in smooth muscle contractility and intestinal fluid dynamics that are induced by IL-4C treatment during a primary infection have a role in its induction of worm expulsion. Changes in smooth muscle contractility are not observed until mice have been treated with IL-4C for 6–7 days; however, decreases in egg production are usually observed within the first 1–3 days of IL-4C treatment. In addition, preliminary experiments suggest that the ability of the worm to feed on host intestinal mucosa is suppressed within 24 hours after the start of IL-4C treatment. Furthermore, 5-LO-deficient mice expel worms (albeit somewhat more slowly than do normal mice) when treated with exogenous IL-4, even though IL-4C treatment of 5-LO-deficient mice does not increase smooth muscle contractility. Expulsion is also delayed, but still induced, when W/W^v mice, infected for the first time with *H. polygyrus*, are treated with IL-4C, even though IL-4-induced increases in smooth muscle contraction and changes in epithelial fluid movement are not observed in these mice. IL-4C treatment also decreases egg production, and to some extent adult worm number, in SCID mice, which fail to respond to IL-4C with increased smooth muscle responsiveness and increased, glucose-induced, intestinal fluid absorption (65, 107; C Sullivan, T Shea-Donohue, manuscript in preparation; JF Urban Jr, FD Finkelman, unpublished data).

Studies of antibody-mediated immunity to *H. polygyrus* also raise questions about the mechanisms by which IL-4 contributes to host protection. Several investigators have demonstrated that considerable protection against a primary *H. polygyrus* infection is afforded naive mice by transfer of large volumes of immune serum (117–121). IgG1, the principal Ig isotype produced in *H. polygyrus*-infected mice, is the principal protective factor in immune serum (119, 120). Unlike treatment with exogenous IL-4, immune serum must be injected during the first few days of infection, when parasite larvae are still encysted within the wall of the small intestine, to be effective (114). Although IL-4 can contribute to the generation of an IgG1 response, treatment with anti-IL-4

receptor mAb at the time of a challenge infection blocks protective immunity without interfering with the polyclonal IgG1 response to the challenge infection (122). Possibly, IL-4 is required to induce a cell type or immune mechanism that interacts with IgG1 antibody to effect expulsion; alternately, IL-4 may be required more for the production of *H. polygyrus*-specific IgG1 antibody than for the polyclonal IgG1 response. It is unlikely that the host-protective role of IL-4 is related to the stimulation of an IgE response, even though IgE responses in *H. polygyrus*-infected mice are IL-4-dependent, because host protection is not blocked by treatment with anti-IgE mAb and is normal in mice that lack the high-affinity IgE receptor (IM Katona, JF Urban Jr, FD Finkelman, unpublished data; JF Urban Jr, D Dombrowicz, JP Kinet, unpublished data).

A role for the humoral immune system in host protection against *H. polygyrus* is shown not only by serum transfer experiments, but also by studies with μMT mice, which lack B cells. These mice develop more severe second infections with *H. polygyrus* than do normal mice of the same genetic background (C57BL/6). It is not known, however, whether this defect reflects an important direct role for antibodies in host protection, or whether B cells and/or antibody enhance antigen presentation and T cell activation in *H. polygyrus*-infected mice. The latter possibility is suggested by a 2–3-fold reduction in cytokine expression in *H. polygyrus*-infected mice and a considerable reduction in the development of mucosal mastocytosis, as compared to normal *H. polygyrus*-infected mice (JF Urban Jr, KB Madden, FD Finkelman, WC Gause, unpublished data). It is unlikely that antibody is the principal mechanism of host protection against *H. polygyrus* infection, because the resistance of different strains of mice to *H. polygyrus* infection does not correlate with the ability of immune serum from these strains to protect naive mice against this parasite (120).

Another possible mechanism by which IL-4 may contribute to immunity against *H. polygyrus* is its induction of VCAM-1 expression. IL-4 induction of VCAM-1 expression by high venous endothelium may have a role in IL-4-dependent immunity against *H. polygyrus*, especially if immunity were directed against larvae in the gut wall rather than against adult worms in the gut lumen, because VCAM-1 expression might be required to allow VLA-4^+ lymphocytes, eosinophils, basophils, and macrophages to enter the vicinity of the larvae. Experiments in which mice were injected with blocking mAbs to both VLA-4 and VCAM-1, however, have failed to inhibit protective immunity to a challenge *H. polygyrus* infection (JF Urban, Jr, FD Finkelman, unpublished data).

STUDIES WITH *N. BRASILIENSIS*-INFECTED RODENTS The relationship between IL-4 and host protection differs between mice infected with *H. polygyrus* and mice infected with *N. brasiliensis*: IL-4 is necessary but not sufficient to completely protect hosts in mice infected with the former parasite, while it is

sufficient but not necessary to protect mice infected with the latter parasite. IL-4-deficient mice expel *N. brasiliensis* normally during a primary infection, but fail to expel *H. polygyrus* during a challenge infection, whereas treatment with exogenous IL-4 completely cures SCID mice infected with *N. brasiliensis* but only partially limits worm survival and fecundity in mice infected with *H. polygyrus* (65). Although a primary *N. brasiliensis* infection induces many of the same changes in intestinal smooth muscle contractility and fluid dynamics that have been observed in mice inoculated for a second time with *H. polygyrus* (48, 123–125; C Sullivan, T Shea-Donohue, unpublished data), dissociation of these physiological responses and IL-4C-induced worm expulsion is even more pronounced in mice infected with *N. brasiliensis*: IL-4C induction of *N. brasiliensis* expulsion is B cell-, T cell-, leukotriene- and mast cell–independent [it is delayed only slightly in anti-*c-kit* mAb-treated SCID mice or 5-lipoxygenase-deficient mice treated with anti-CD4 mAb, as compared to SCID mice treated with a control mAb or normal mice treated with anti-CD4 mAb (JF Urban Jr, C Funk, FD Finkelman, unpublished data)].

The lack of mast cell involvement in IL-4C-induced *N. brasiliensis* expulsion from anti-CD4 mAb-treated mice and SCID mice is consistent with observations that mast cells have little involvement in naturally occurring *N. brasiliensis* expulsion. Expulsion of *N. brasiliensis* from the gut of W/W^v mice has been described as slow in some, but not all, studies (126, 127). Studies in which expulsion was slow reported that bone marrow reconstitution, which restores mast cells, did not correct for slow expulsion from the gut. This suggests that any defect in *N. brasiliensis* expulsion by W/W^v mice might result from the abnormal intestinal pacemaker activity in these mice (112), which, because it develops as a result of absent *c-kit* activity during the neonatal period, would not be corrected by bone marrow reconstitution, or it might develop from the absence of intraepithelial $\gamma\delta$ T cells in W/W^v mice. This $\gamma\delta$ T cell defect may affect intestinal secretory responses in *N. brasiliensis*–infected mice, because the intestinal secretory response to cholera toxin is deficient in W/W^v mice and is not correctable by reconstitution with bone marrow (128). Studies with *N. brasiliensis*-infected rats support the view that mast cells are not important for controlling infections with this parasite, and they even raise the possibility that mast cell products may contribute to parasite fecundity: Treatment of infected rats with anti–stem cell factor antiserum decreases mucosal mast cell number and parasite egg production in *N. brasiliensis*-infected rats, while treatment with stem cell factor enhances mast cell activity early in infection, but increases parasite egg production (129).

In contrast to these observations with *N. brasiliensis*-infected rodents, the gastrointestinal nematodes *S. ratti* and *S. venezuelensis* are expelled considerably more slowly by W/W^v mice than by normal mice, and delayed expulsion is

corrected by reconstituting W/W^v mice with normal bone marrow (130, 131). In addition, induction of mucosal mastocytosis by treatment of normal mice with IL-3 protects mice against infection with *Strongyloides ratti* but not against infection with *N. brasiliensis*. W/W^v mice, which do not develop intestinal mucosal mastocytosis when treated with IL-3, do not develop resistance against *S. ratti* in response to IL-3 treatment (132). These observations are all consistent with the interpretation that mucosal mast cells have more of a role in host protection against *Strongyloides* sp. than against *N. brasiliensis*.

The ability of IL-4C to cause *N. brasiliensis* expulsion in the absence of mast cells, leukotrienes, or the specific immune system raises the possibility that IL-4 might act directly on this worm rather than indirectly damage the worm through its actions on the host. Two sets of experiments demonstrate that this is not the case: First, a rat anti-IL-4 receptor mAb, which binds to the mouse but not to the rat IL-4 receptor, blocks the ability of IL-4C to induce expulsion of *N. brasiliensis* from anti-CD4 mAb-treated mice (65). Even if *N. brasiliensis* had, during its evolution, acquired the gene for a mammalian IL-4 receptor, it should have acquired the gene of its natural host, the rat, and, hence, expressed a receptor that is not blocked by the mAb used in these experiments. Second, IL-4C fails to induce the expulsion of *N. brasiliensis* from anti-CD4 mAb-treated mice that are defective for the IL-4 signal transduction molecule Stat6 (JF Urban Jr, FD Finkelman, unpublished data). If IL-4 acted directly on the worm, an IL-4 signal transduction defect in the host should not have affected IL-4C induction of expulsion.

As was true with *H. polygyrus* infections, the effects of exogenous IL-4 treatment on the host promote expulsion of adult *N. brasiliensis*, rather than killing of larvae. Although it is not yet known whether IL-4 interferes with worm nutrition, two observations point in this direction: 1. As was seen in *H. polygyrus* infections, IL-4C treatment has a slow effect on *N. brasiliensis*, first causing a decrease in fecundity, and later, caudal migration and eventual expulsion (65). And 2. *N. brasiliensis* adults are still alive, albeit smaller and paler than normal when expelled, and they can regain vitality and reestablish infection when transferred by oral gavage to naive, untreated mice (133). The IL-4-induced increase in intestinal permeability may inhibit feeding by blocking worm contact with the gut mucosa and may represent a more chronic variant of the marked protein leak from villar capillaries that is associated with rapid expulsion of *N. brasiliensis* from the gut of rats that are undergoing an anaphylactic reaction (134).

The IL-4-independent mechanism(s) that induce *N. brasiliensis* expulsion has not been identified but is known to be $CD4^+$ T cell–dependent and suppressible by interferon-γ and interferon-α/β (50, 135). Possible candidate inducing factors and mechanisms include cytokines other than IL-4, mucus trapping, antibody-mediated worm damage, and lipid peroxidation.

Other cytokines In addition to IL-4, cytokines that have been studied for possible involvement in expulsion of *N. brasiliensis* include IL-3, IL-5, IL-6, IL-9, and IL-13. Although IL-13 shares many of the effects of IL-4 (136), we initially thought it unlikely that IL-13 mediates IL-4-independent expulsion of *N. brasiliensis*, because expulsion is not inhibited by anti-IL-4 receptor mAb, which blocks the IL-13 receptor as well as the IL-4 receptor (RA Morawetz, L Gabriele, LV Rizzo, N Noben-Trauth, R Kühn, K Rajewsky, W Müller, TM Doherty, F Finkelman, RL Coffman, HC Morse III, "IL-4-independent immunoglobulin class switch to IgE in the mouse," submitted for publication). However, a recent experiment, which demonstrates that Stat6 KO mice fail to expel *N. brasiliensis* (JF Urban Jr, SC Morris, KB Madden, JN Ihle, FD Finkelman, unpublished observation), makes it quite likely that IL-13 is responsible for IL-4-independent expulsion of this parasite, because Stat6 is only known to be involved in IL-4 receptor and IL-13 receptor signal transduction. Thus, either IL-13 can induce mice to expel *N. brasiliensis* (in which case anti-IL-4 receptor antibody must block the IL-13 receptor less effectively than it blocks the IL-4 receptor), or *N. brasiliensis* can be induced by another, still unidentified cytokine that signals via Stat6.

Currently there is little evidence that cytokines other than IL-4 and IL-13 induce *N. brasiliensis* expulson. Treatment of BALB/c mice with antibodies to IL-6 or with anti-IL-4 plus anti-IL-5 [which nearly completely suppresses eosinophilia and IgE production (122)], or anti-IL-3 plus anti-IL-4, and anti-IL-9 [which nearly completely suppresses mucosal mastocytosis and IgE production (KB Madden, JF Urban Jr, A Svetic′, WC Gause, FD Finkelman, IM Katona, "The role of cytokines in helminth-induced mucosal mast cell hyperplasia," in preparation)], also fail to inhibit *N. brasiliensis* expulsion.

Mucus trapping *N. brasiliensis* expulsion is accompanied by an increase in intestinal goblet cell number and mucus production, as well as by a change in the carbohydrate content of secreted mucus (137). These changes are at least relatively T cell–dependent (48) and IL-4–independent (KB Madden, JF Urban Jr, FD Finkelman, unpublished data) and may favor expulsion by trapping worms, preventing them from adhering to the gut or feeding. However, extensive studies of mucus trapping of *T. spiralis* in infected rats have shown that this phenomenon is not critical for parasite expulsion (138), and no studies have been performed that test whether selective elimination of mucus production affects *N. brasiliensis* expulsion.

Antibody-mediated protection Serum from immune mice provides protection against *N. brasiliensis* (139). While anti-IgM-suppressed mice, which produce little antibody, are able to expel *N. brasiliensis* (43), it remains possible that either antibody or IL-4 can induce expulsion, so that only mice that have neither

will develop chronic infections. Antibodies do not need to promote killing mechanisms to provide host protection. Antibodies can participate in mast cell activation, which leads to changes in gut physiology, and may block parasite receptors that might promote adhesion to the gut mucosa. In addition, mucus trapping has been demonstrated in *T. spiralis*-infected rats to be an antibody-dependent process (140).

Lipid peroxidation The possibility that lipid peroxidation by host-produced reactive oxygen intermediates damages *N. brasiliensis* and leads to its expulsion was suggested by reports that increased peroxidation of gut lipids is observed at the time of *N. brasiliensis* expulsion and that butylated hydroxyanisole, which scavenges reactive oxygen intermediates, suppresses the expulsion process (butylated hydroxyanisole, however, has additional metabolic effects) (141, 142). The existence of a process that expels nematodes through the production of reactive oxygen intermediates could provide an evolutionary explanation for why nematodes produce enzymes, such as catalase, glutathione reductase, and superoxide dismutase, that offer some protection against reactive oxygen intermediates (143). Lipid peroxidation, as a mechanism for inducing worm expulsion, was potentially linked to IL-4 by the observation that IL-4 stimulates, in humans, production of the lipid peroxidating enzyme 15-lipoxygenase (144, 145). Human 15-lipoxygenase is highly toxic for at least some helminths; it kills schistosomula larvae of *Schistosoma mansoni* in vitro with a potency at least 10-fold greater than that of eosinophil basic protein (A Mahmoud, personal communication). Mice lack 15-lipoxygenase, but express a homologous enzyme, 12-lipoxygenase (146, 147). Further studies, however, demonstrated that normal mice and IL-4 KO mice have equivalent levels of 12-lipoxygenase, and that in vivo treatment of mice with IL-4 fails to induce increased 12-lipoxygenase expression (J Cornicelli, JF Urban Jr, FD Finkelman, unpublished data). Thus, while the possibility remains that lipid peroxidation may be involved with the induction of *N. brasiliensis* expulsion, there is currently no way to associate this in mice with IL-4.

STUDIES WITH *T. MURIS* *T. muris* resembles *H. polygyrus* rather than *N. brasiliensis* in that it is an entirely enteral infection and its expulsion is blocked by anti-IL-4 receptor antibody. Both IL-4 and IL-9 [which can enhance IL-4 effects (103, 104)] promote expulsion of *T. muris* by normally susceptible mouse strains (56; HC Faulkner, RM Grencis, personal communication). In this regard, it is of interest that IL-9 and IL-4 share a signaling pathway: Both tyrosine phosphorylate the insulin receptor substrate-1 molecule but differ in their signaling through Stat molecules (IL-4 tyrosine phosphorylates Stat6, whereas IL-9 tyrosine phosphorylates Stat3) (148). As with the other parasites discussed here, specific antibodies can promote, but are not necessary to induce,

expulsion (149, 150). Unlike *T. spiralis*, anti-*c-kit* mAb does not appear to prevent expulsion (LE Donaldson, KJ Else, RM Grencis, personal communication). Normally resistant mice that have been treated with anti-IL-3 mAb still expel *T. muris* effectively, even though their mast cell responses are depressed (KJ Else, RM Grencis, unpublished data). The mechanisms by which IL-4 (or IL-13) contributes to expulsion of this parasite remain largely uninvestigated.

GENERAL CONCLUSIONS

Studies of four different gastrointestinal nematode parasites have shown that expulsion of each is dependent upon $CD4^+$ T cells, promoted by IL-4, and in at least some cases inhibited by IFNs. Control of two of these parasites (*T. muris* and *H. polygyrus*) appears to be highly IL-4-dependent, while IL-4 induces redundant protection for a third (*N. brasiliensis*) and decreases the intensity of infection with the fourth (*T. spiralis*). IgE/mast cell–mediated mechanisms are likely to be central to the rapid expulsion that occurs in immune rats challenged with *T. spiralis* larvae; however, neither this nor IL-4-dependence of the rapid expulsion phenomenon has been demonstrated directly. The immune mechanisms that are responsible for expulsion of the other parasites, or for expulsion of *T. spiralis* from mice or from rats that harbor a primary infection, are even less well understood. In instances where IL-4C treatment promotes a slow decrease in worm fecundity and eventually induces expulsion, it seems likely that a mechanism that interferes with worm ingestion of food is involved, but the nature of this mechanism is not clear. There is strong evidence that IL-4 promotes intestinal mucosal mastocytosis, IgE production, and in some cases, IgG1 production, and stimulates a change in intestinal fluid dynamics that favors fluid accumulation in the gut lumen and an increase in small intestine smooth muscle contractility; however, none of these phenomena has been clearly demonstrated to be required for expulsion. Thus, host protection against parasitic gastrointestinal nematodes appears often to be more dependent upon effects of type 2 cytokines, in general, and IL-4, in particular, that are still poorly characterized, than on the classic type 2 cytokine-dependent, worm infection–related triad of eosinophilia, mastocytosis, and IgE production.

Further experiments with Stat6 KO mice should be useful for testing the relevance of IL-4–induced physiological events to worm expulsion. Although Stat6 KO mice do not respond to IL-4 with increases in either class II MHC or CD23 expression and although they are unable to make IgE responses, IL-4 can act as a growth factor for B and T cells from these mice (151, 152) and can induce a large mucosal mast cell response in anti-CD4 mAb-treated *N. brasiliensis*-infected mice (JF Urban, Jr, KB Madden, FD Finkelman, unpublished data). Thus, mast cell–dependent changes in gut physiology may still occur in Stat6 KO mice.

Although some IL-4-induced phenomena that are involved in worm expulsion may remain undiscovered, the failure to identify unique mechanisms that are responsible for the expulsion of specific parasitic nematodes may reflect redundancy of host defenses. Inhibition of a single redundant defense mechanism may have no detectable effect on worm expulsion or may merely retard expulsion. Host defense mechanisms against parasitic nematodes may be redundant at two levels: First, a single cytokine, IL-4, most likely induces multiple effects, more than one of which may induce worm expulsion; and second, for at least some parasitic nematodes, IL-4 is only one of multiple stimuli that can induce worm expulsion. In some instances this redundancy may be limited to induction of a single signal transduction pathway by more than one stimulus, but in other instances, totally unrelated defense mechanisms appear able to control infection. The redundancy of host defense mechanisms against parasitic nematodes may seem inefficient, and it probably subjects the host to untoward effects of defense mechanisms that are unnecessary to expel a particular parasite. The adaptive nature of redundancy makes biological sense, however, for two reasons. One is that just as natural selection promotes the evolution of host defenses against parasites, it promotes parasite evolution of mechanisms that evade host defenses. Because it would be less likely for a parasite simultaneously to develop means of evading several defense mechanisms, the employment of redundant defenses against a particular parasite should inhibit the selection of parasites that resist any of these defenses, just as treating bacterial infections simultaneously with multiple antibiotics inhibits the selection of bacteria that resist any of the antibiotics. Host deployment of redundant defenses also makes sense if the host has a limited ability to recognize distinct features of particular parasites but can recognize a feature that is common to a particular class of parasites.

This situation could cause a set of defense mechanisms to become linked through natural selection if infection by most members of the parasite class can be controlled by at least one mechanism in the set, but no single mechanism in the set can control infection by most of the parasites. By this logic, IL-5 production and eosinophilia, or IL-3 production and mucosal mastocytosis, are induced in mice infected by *N. brasiliensis* not because they protect the host against this particular parasite, but because the host recognizes some feature(s) of *N. brasiliensis* that is common to a class of parasites that includes some members susceptible to attack by eosinophils or mucosal mast cells. Recognition of putative features common to gastrointestinal nematodes presumably informs the host immune system that a stereotypic type 2 cytokine response will be more protective than a type 1 or type 0 cytokine response. However, the recognized features are likely to be too general to allow the host's immune system to safely make only those type 2 responses that protect against a specific

parasite. We hypothesize that some of these parasite features are likely to be shared by strong allergens and may be responsible for the obviously maladaptive responses made to nonthreatening molecules such as dust mite proteases, bee venom phospholipids, or pollen antigens.

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